

SOME OBSERVATIONS CONCERNING THE TRANSMISSION
OF EAST COAST FEVER BY TICKS.

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(Read August 16, 1911.)

It has been proved that *Theileria parva*, the cause of East Coast Fever, can be transmitted by at least five different species of ticks of the genus *Rhipicephalus* (viz., *Rh. appendiculatus*, *Rh. evertsi*, *Rh. nitens*, and *Rh. capensis*). Of these, *Rh. appendiculatus* is the commonest in South Africa and in the regions in which East Coast Fever is known generally (Uganda, British, and German East Africa). They were almost exclusively used in my experience on East Coast Fever.

The following observations, which were made in connection with experiments undertaken for other purposes (mainly to get material for the artificial transmission of the disease, and to test the resulting immunity), form an interesting addition to and confirmation of facts which have already been established concerning the transmission of East Coast Fever by means of ticks.

EXPERIMENT No. 1.

“A.” TO DEMONSTRATE THE FACT THAT BROWN TICK IMAGINES, WHICH AS LARVÆ HAD BECOME INFECTED WITH EAST COAST FEVER, AND HAD TRANSMITTED THE DISEASE IN THEIR NYMPHAL STAGE, ARE NO LONGER INFECTIVE FOR SUSCEPTIBLE CATTLE.

(a) RHIPICEPHALUS APPENDICULATUS, imago (Ref. No. 268).

Note.—The adult females originated from Natal on January 12, 1910; the eggs were laid on January 18, 1910, and hatched into larvæ in the laboratory on February 18, 1910.

Infecting the Ticks.—The larvæ were fed on Heifer 923 from March 20 to 23, 1910, that is, from the 20th to 23rd day of the disease, and at a

time when the parasites of East Coast Fever were very frequent in the blood.

Cleaning the Ticks.—The larvæ moulted in due time, and the nymphæ were placed on Cow 596 on April 7, 1910. Cow 596 developed the disease after an incubation time of 15 days, and was killed for experimental purposes on the 12th day of the East Coast Fever illness.

Remarks.—The nymphæ collected as engorged larvæ off Heifer 923 whilst undergoing an East Coast Fever reaction proved to be infective for Cow 596.

Note.—The nymphæ which communicated the disease to Cow 596 engorged and were collected off this cow from April 13 to 15, 1910. They moulted into adults on May 20, 1910, and were used for the infestation of cattle Nos. 1022 and 1060 (*vide* Experiment No. 1 "C," Nos. 21 and 22).

(b) *RHIPICEPHALUS APPENDICULATUS*, imagines (Ref. No. 309).

Note.—The engorged females originated from Natal on December 9, 1909. The eggs were laid on December 24, 1909, and the larvæ hatched out on January 30, 1910.

Infecting the Ticks.—The larvæ were fed on Calf 700 from February 27, 1910, and dropped between March 2nd and 7th, that is, from the 7th and 8th days of the disease, and at a time when *Theileria parva* were frequently met with in the blood.

Cleaning the Ticks.—The engorged larvæ moulted on March 17, 1910, and the nymphæ were placed on Calf 917 on March 30, 1910. This calf developed East Coast Fever after the usual period and died; the examination of the blood proved the presence of *Theileria parva*, and the glands contained Agamonts and Gamonts in large numbers.

Remarks.—The ticks which as larvæ were fed on Calf 700 during the time of the East Coast Fever reaction proved virulent for Calf 917 in their nymphal stage.

Note.—The nymphæ which communicated the disease to Calf 917 engorged and were collected from April 3 to 7, 1910, namely, a week after infestation. They moulted into adults on May 4, 1910, and were placed on Heifer 1088 on December 15, 1910, and on Calf 1145 on January 6, 1911, in order to test their infectivity (*vide* later Experiment No. 1 "C," Nos. 23 and 24).

(c) *RHIPICEPHALUS APPENDICULATUS*, imagines (Ref. No. 335).

Infecting the Ticks.—The larvæ which originated from females collected in Natal on December 19, 1909; the eggs were laid on December 24, 1909, and the larvæ hatched out on January 30, 1910. On February

27, 1910, the larvæ were placed on Calf 917. (Calf 917 contracted East Coast Fever from the ticks of Calf 700—*vide* sub-head (b).) The larvæ were collected off Calf 917 between April 11 and 14, 1910, at a time when *Theileria parva* were frequently met with in the blood and the plasma bodies were present in the organs.

Cleaning the Ticks.—The engorged larvæ moulted in due time, together with nymphæ off Cattle 700 (Ref. No. 309) and off 923 (Ref. No. 268) were used for the infestation of the following eight cattle: Nos. 561, 908, 919, 1101, 914, 1012, 1026, and 1040.

(c) RHIPICEPHALUS APPENDICULATUS (Ref. Nos. 309, 268, and 335).

1. Heifer 561 was infected on July 26, 1910, with brown nymphæ as above, and developed East Coast Fever after an incubation period of 12 days. This heifer was killed for experimental purposes 10 days later, when microscopical examination proved the presence of *Th. parva* in the blood and the plasma bodies in the glands.

Remarks.—The ticks which as larvæ had fed on Cattle 700, 917, and 923, proved virulent in their nymphal stage for Heifer 561.

Note.—The engorged brown nymphæ were all collected by August 2, 1910. They moulted into adults on September 19, 1910, and were placed on Heifer 1088 on December 15, 1910, and on Calf 1145 on January 6, 1911 (*vide* Experiment 1 "C," Nos. 23 and 24).

2. Heifer 908 was infested on July 26, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 355), as above. This heifer developed East Coast Fever after an incubation time of 13 days, and was killed for experimental purposes on August 22, 1910, after an illness of 14 days. The blood contained *Th. parva*, and in the glands and spleen plasma granules were frequently found.

Note.—The engorged brown nymphæ were collected by August 2, 1910, and had moulted into adults on September 19, 1910; the adults were placed on Heifer 1088 on December 15, 1910, and on Calf 1145 on January 6, 1911, in order to test their infectivity (*vide* later Experiment No. 1 "C," Nos. 23 and 24).

3. Calf 919 was infested on June 27, 1910, with brown nymphæ off Cattle 700, 917, and 923 (Ref. Nos. 309, 335, and 268), as above. An East Coast Fever reaction set in after an incubation time of 14 days, and the calf died on July 28, 1910, the 16th day of illness. The blood contained *Th. parva* in large numbers, and plasma bodies were frequently met with in the glands.

Note.—The engorged brown nymphæ were all collected by July 12, 1910, and moulted into adults in due time, when they were placed on

Heifer 1088 and Calf 1145 in order to test their infectivity (*vide* later Experiment No. 1 "C," Nos. 23 and 24).

4. Heifer 914 was infested on June 28, 1910, with brown nymphæ off Cattle 700, 917, and 923 (Ref. Nos. 309, 335, and 268), as above. It developed East Coast Fever after an incubation time of 13 days and recovered. The blood contained *Th. parva*, and plasma bodies were found in the glands.

Note.—The engorged brown nymphæ were all collected by July 12, 1910, and moulted into adults by September 19, 1910; they were placed on Heifer 1088 and Calf 1145 on December 15, 1910, and on January 6, 1911, respectively, in order to test their infectivity (*vide* later Experiment No. 1 "C," Nos. 23 and 24).

5. Heifer 1012 was infested on June 27, 1910, with brown nymphæ off Cattle 700, 917, and 923 (Ref. Nos. 309, 335, and 268), as above. It developed East Coast Fever after an incubation time of 12 days, and died on July 26, 1910. The blood was found to contain *Th. parva*, and plasma bodies were noted in the glands.

Note.—The engorged nymphæ were collected in due time, and were finally placed on Heifer 1088 and on Calf 1145 in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 23 and 24).

6. Heifer 1011 was infested on June 27, 1910, with brown nymphæ off Cattle 700, 917, and 923 (Ref. Nos. 309, 335, and 268), as above. After an incubation time of 13 days an East Coast Fever reaction set in, and the heifer was killed for experimental purpose on the 8th day of the disease (July 18, 1910). The blood contained *Th. parva* in rare numbers, but plasma bodies were found to be very frequent in the glands.

Note.—The engorged nymphæ were collected in due time, and had moulted by September 19, 1910. They were placed on Heifer 1088 on December 15, 1910, and on Calf 1145 on January 6, 1911, in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 23 and 24).

7. Ox 1026 was infested on June 27, 1910, with brown nymphæ off Cattle 700, 917, and 923 (Ref. Nos. 309, 335, and 268), as above. East Coast Fever developed after an incubation time of 12 days, and the ox was killed on the 16th day of illness for experimental purposes (July 25, 1910). The blood contained *Th. parva*, and the glands showed the presence of plasma bodies in large numbers.

Note.—The engorged nymphæ were collected in due time, and moulted into adults by September 19, 1910; they were used for the infestation of Heifer 1088 and Calf 1145 on December 15, 1910, and January 6, 1911,

respectively, in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 23 and 24).

8. Ox 1040 was infested on June 27, 1910, with brown nymphæ off Cattle 700, 917, and 923 (Ref. Nos. 309, 335, and 268), as above. Ox 1040 developed East Coast Fever after an incubation time of 13 days, and died on the 15th day of illness (July 25, 1910). The blood showed a strong infection of *Th. parva*, but plasma bodies were found in rare numbers only in the glands.

Note.—The nymphæ engorged in due time, and moulted into adults by September 19, 1910; they were used for the infestation of Heifer 1088 and Calf 1145 (*vide* Experiment No. 1 "C," Nos. 23 and 24).

EXPERIMENT No. 1 (*continued*).

"B." TO DEMONSTRATE THE FACT THAT BROWN TICK IMAGINES, WHICH AS LARVÆ HAD BECOME INFECTED WITH EAST COAST FEVER, AFTER HAVING PASSED THEIR NYMPHAL STAGE ON CATTLE RENDERED IMMUNE TO EAST COAST FEVER BY INOCULATION, NO LONGER TRANSMIT THE DISEASE TO SUSCEPTIBLE CATTLE.

(e) RHIPICEPHALUS APPENDICULATUS (Ref. Nos. 309, 268, and 335).

(See previous sub-head (*d*).)

Note.—The animals referred to later (Nos. 829, 883, 836, 895, 1047, 1033, 871, 621, 679, and 615) had all been rendered immune to East Coast Fever by inoculation, details of which were given in the Annual Report of the Government Veterinary Bacteriologist for 1909–10, under the title of "The Artificial Transmission of East Coast Fever" (see pp. 1–55).

9. Ox 829 was rendered immune to East Coast Fever by inoculation (see above).

Infested on March 24, 1910, with brown nymphæ off Calf 700 (Ref. No. 309).

Note.—The engorged nymphæ were collected on March 30, 1910; they moulted into adults on April 21, 1910, and were placed on Heifers 1057 and 1058 on August 5, 1910, in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 25 and 26).

10. Heifer 883 was rendered immune by inoculation (*vide* above).

Infested on March 24, 1910, with brown nymphæ off Calf 700 (Ref. No. 309).

Note.—The engorged nymphæ were collected on March 30, 1910; they moulted into adults on April 21, 1910, and in their adult stage were used for the infestation of Heifers 1057 and 1058 (*vide* Experiment No. 1 “C,” Nos. 25 and 26), on August 5, 1910, in order to test their infectivity.

11. Heifer 836 rendered immune by inoculation (*vide* above).

Infested on March 24, 1910, with brown nymphæ off Calf 700 (Ref. No. 309).

Note.—The engorged nymphæ dropped on March 30, 1910, and moulted into adults on April 21, 1910; the adults were placed on Heifers 1057 and 1058 on August 5, 1910, in order to test their infectivity (*vide* Experiment No. 1 “C,” Nos. 25 and 26).

12. Heifer 895 was rendered immune by inoculation (*vide* above).

Infested on March 24, 1910, with brown nymphæ off Calf 700 (Ref. No. 309).

Note.—The engorged nymphæ dropped on March 30, 1910, and moulted into adults on April 21, 1910; they were placed on Heifers 1057 and 1058 on August 5, 1910, in order to test their infectivity (*vide* Experiment No. 1 “C,” Nos. 25 and 26).

13. Ox 1047 rendered immune by inoculation (*vide* above).

Infested on July 28, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected on August 2, 1910, and moulted into adults on September 19, 1910; the adults were used for the infestation of Heifer 1088 and Calf 1145 (*vide* Experiment No. 1 “C,” Nos. 25 and 26) in order to test their infectivity.

14. Cow 1033 was rendered immune by inoculation (*vide* above).

Infested on July 27, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected on August 2, 1910, and moulted into adults on September 19, 1910; later they were placed on Cattle 1088 and 1145 (*vide* Experiment No. 1 “C,” Nos. 25 and 26) in order to test their infectivity.

15. Heifer 871 was rendered immune by inoculation (*vide* above).

Infested on June 28, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected and moulted into adults in due time; later they were used for the infestation of Cattle 1088 and

1145 in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 25 and 26).

16. Ox 621 was rendered immune by inoculation (*vide* above).

Infested on July 2, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected within the usual time, and moulted into adults on September 19, 1910; the adults were later placed on Cattle 1088 and 1145 to test their infectivity (*vide* Experiment No. 1 "C," Nos. 25 and 26).

17. Heifer 895 was rendered immune by inoculation.

Infested on July 2, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected from July 4 to 14, 1910, and moulted into adults on September 19, 1910; they were later placed on Cattle 1088 and 1145 in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 25 and 26).

18. Heifer 836 was rendered immune by inoculation (*vide* above).

Infested on July 2, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected from July 4 to 14, 1910, and moulted into adults on September 19, 1910; they were later placed on Cattle 1088 and 1145 in order to test their infectivity (*vide* Experiment No. 1 "C," Nos. 25 and 26).

19. Cow 679 was rendered immune by inoculation (*vide* above).

Infested on July 2, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected from July 4 to 14, 1910, and moulted into adults on September 19, 1910. In order to test their infectivity they were placed on Cattle 1088 and 1145 (*vide* Experiment No. 1 "C," Nos. 25 and 26).

20. Bull rendered immune by inoculation (*vide* above).

Infested on July 2, 1910, with brown nymphæ off Cattle 700, 923, and 917 (Ref. Nos. 309, 268, and 335).

Note.—The engorged nymphæ were collected from July 4 to 14, 1910, and moulted into adults on September 19, 1910; later in order to test infectivity they were placed on Cattle 1088 and 1145 (*vide* Experiment No. 1 "C," Nos. 25 and 26).

EXPERIMENT No. 1 (*continued*).

"C." TO SHOW THAT THE TICKS WHICH AS NYMPHÆ HAD COMMUNICATED THE DISEASE TO SUSCEPTIBLE CATTLE (Nos. 596, 561, 908, 919, 914, 1012, 1026, AND 1040), OR WHICH HAD PASSED THEIR NYMPHAL STAGE ON ANIMALS ARTIFICIALLY IMMUNISED (Nos. 829, 883, 836, 895, 1047, 1033, 871, 621, 895, 836, 679, AND 615) DO NOT TRANSMIT THE DISEASE IN THEIR ADULT STAGE TO SUSCEPTIBLE CATTLE.

(*f*) RHIPICEPHALUS APPENDICULATUS (Ref. No. 268).

Note.—The following Cattle Nos. 1022, 1060, 1088, 1045, 1057, and 1058 were all born and bred in areas free from East Coast Fever, and accordingly were susceptible to the disease.

21. Heifer 1022 was infested on June 23, 1910, with brown adults off Heifer 923 (Ref. No. 268). This infestation was repeated on June 28th, July 15th, July 20th, July 28th, August 3rd, and August 20th. Altogether 100 ticks were placed on this heifer, but not all of them became attached.

Remarks.—This animal remained quite healthy.

22. Heifer 1060 was infested on August 29, 1910, with brown adults of Heifer 923 (Ref. No. 268), and was re-infested on September 14, 1910; 8 ticks of the first infestation were counted to be fast and 18 of the second lot.

Remarks.—This heifer remained healthy. Later she was used for inoculation purposes, and was finally exposed to East Coast Fever infection on the farm "Burnside," Natal, and died on the 21st day of East Coast Fever (*vide* article on "Progress Report on the Possibility of Vaccinating Cattle against East Coast Fever," Experiment 6 "D").

23. Heifer 1088 was infested on December 15, 1910, with a large number of brown adults which as nymphæ had given the disease to Cattle Nos. 561, 908, 919, 914, 917, 1011, 1012, 1026, 1040, or which as nymphæ had fed on immune Cattle Nos. 1047, 1033, 871, 621, 895, 836, 679, 615, 829, and 883; the ticks were fast on December 16, 1910.

Remarks.—Heifer 1088 did not contract East Coast Fever from this infestation, but was used later for inoculation purposes (*vide* article cited above, Experiment 9 "J"); finally was exposed at "Burnside" and died of East Coast Fever complicated with Redwater.

24. Calf 1145 was infested on January 6, 1911, with large numbers of brown adults off Cattle Nos. 561, 908, 909, 914, 917, 1011, 1012, 1026, 1040, 1047, 1033, 871, 621, 895, 836, 679, 615, 883, and 829.

Remarks.—The calf did not contract East Coast Fever from this infestation, but died from Enteritis on February 14, 1911.

25. Heifer 1057 was infested on August 5, 1910, with large numbers of adults which had passed their nymphal stage on Cattle 829, 883, 836, and 895 (Ref. No. 309); she was re-infested on August 19th and 29th with ticks of the same batch. A good many ticks were found fast the following day. The engorged females dropped on August 13th and 23rd, and September 5th.

Remarks.—Heifer 1057 did not contract East Coast Fever from this infestation. It was used later for inoculation experiments (*vide* article quoted above, Experiment 6 “B”).

26. Heifer 1058 was infested on August 5, 1910, with large numbers of brown adults off Cattle 829, 883, 836, and 895 (Ref. No. 309). The ticks were found to be fast the following day, and large numbers of engorged females were collected from August 23rd.

Remarks.—Heifer 1058 did not contract East Coast Fever from this inoculation. It was used later for inoculation experiments (*vide* article cited above, Experiment 6 “K”) finally exposed in “Burnside” and died of East Coast Fever. Plasma bodies were found in the blood, spleen, and glands on post-mortem examination.

Results.—None of the six animals which were infected with “cleaned” ticks developed East Coast Fever from the infestation.

CONCLUSIONS.

The adult ticks which as larvæ had fed on sick animals, and which as nymphæ had communicated the disease to nine susceptible cattle, or which had been feeding on twelve artificially immunised cattle, did not communicate East Coast Fever when placed in large numbers on six susceptible cattle.

These observations were made on a fair number of animals and with a great many ticks, so that all coincidences can be excluded.

EXPERIMENT No. 2.

TO SHOW THAT TICKS WHICH HAVE BEEN FEEDING ON CATTLE SUFFERING FROM EAST COAST FEVER DO NOT ALWAYS TRANSMIT THE DISEASE IN THEIR SUBSEQUENT STAGE.

Particulars of the Ticks used.

I.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 319).

The larvæ originated from mothers collected in Natal on March 18, 1910; the eggs were laid in the laboratory on March 25th and the larvæ

hatched out on April 26, 1910. The larvæ were placed on Heifer 1013 on the 7th day of the fever reaction (May 30, 1910), and were collected during the three days previous to death, which occurred from East Coast Fever on June 7, 1910; during the time the ticks were feeding on Heifer 1013 the parasites of East Coast Fever were frequently met with, and all stages of development were found in the lymphatic glands.

The larvæ moulted into nymphæ on August 5, 1910.

II.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 364).

The larvæ originated from mothers collected in Natal on March 18, 1910; the eggs were laid at the laboratory on March 25th, and the larvæ hatched out on April 26, 1910. The larvæ were placed on Heifer 908 on August 12, 1910, viz., the 5th day after the rise of temperature, and were collected 5 days before death, which occurred on August 22, 1910, during which period the blood showed *Theileria parva* in large numbers, and in the spleen and glands plasma bodies were very frequently met with.

The larvæ moulted into nymphæ on September 10, 1910.

III.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 355).

The larvæ originated from mothers collected in Natal on March 18, 1910, and the eggs were laid in the laboratory on March 25; the larvæ hatched out on April 26, 1910. The larvæ were placed on Heifer 913 on July 14, 1910, at a time when the parasites of East Coast Fever were frequently met with, and the engorged larvæ were collected from July 19 to 21, 1910. Heifer 913 was killed on July 22, 1910. The examination of the blood since July 14th had shown the presence of the East Coast Fever parasites in but rare numbers; free plasma bodies (agamogonous forms) had been noted in the glands.

The larvæ moulted into nymphæ on September 1, 1910.

IV.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 356).

The larvæ originated from mothers collected in Natal on March 12, 1910. The eggs were laid at the laboratory on March 25th, and the larvæ emerged on April 18, 1910. The larvæ were placed on Heifer 914 on July 14, 1910, and were collected from July 17th to 20th. On July 18th *Th. parva* were noted in the blood of Heifer 914, and plasma bodies were seen in the glands. The heifer recovered.

The engorged larvæ moulted into nymphæ on September 15, 1910.

V.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 349).

The engorged nymphæ were collected off sick cattle in Natal on June 29, 1910, and moulted in the laboratory on September 11, 1910.

VI.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 411).

The larvæ originated from mothers collected in Natal on March 12, 1910. The eggs were laid at the laboratory on March 14, 1910, and the larvæ hatched on April 18, 1910. The larvæ were placed on Heifer 914, suffering from East Coast Fever, on July 14, 1910. The engorged larvæ were collected from July 17th to 20th and moulted into nymphæ on August 15, 1910; they were placed on Heifer 1053 on October 18, 1910, at a time when this heifer was suffering from East Coast Fever and when *Th. parva* were noted in the blood and plasma bodies were seen in the glands. The engorged nymphæ dropped from October 22 to 27, 1910, and moulted into adults on November 21, 1910.

Note.—These ticks had been feeding in both their larval and nymphal stages on animals suffering from East Coast Fever.

VII.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 426).

The larvæ originated from mothers collected in Natal on February 18, 1910, and hatched by March 30, 1910; the larvæ were reared on "clean" Heifer 868 and dropped from July 12 to 15, 1910; they moulted on August 23, 1910, and the nymphæ were placed on Heifer 1111 on October 26th at a time when this heifer was suffering from East Coast Fever. *Th. parva* being found in the blood and plasma bodies in the glands. The engorged nymphæ dropped from October 31 to November 5, 1910, and moulted into adults on November 26, 1910.

VIII.

RHIPICEPHALUS APPENDICULATUS (Ref. No. 373).

The adult females originated from Cattle 906, 1088, 1009, and 1021, animals which were kept at the laboratory. The eggs hatched on November 18, 1910, and the larvæ were placed on Heifer 909 on January 2, 1911, at a time when this heifer was undergoing an attack of East Coast Fever and when *Th. parva* and plasma bodies were noted in the blood and glands respectively.

The engorged larvæ were collected from January 5 to 7, 1911, and moulted about January 20, 1911, into nymphæ.

Testing of the Ticks.

(a) BROWN NYMPHÆ (Ref. Nos. 363, 364, 355, 356, and 349).

1. Heifer 627 had previously been used for inoculation experiments in connection with the transmission of East Coast Fever, but with negative results.

Infested with brown nymphæ (Ref. No. 363), on August 17, 1910; about 20 were found attached the following day, and the engorged nymphæ were collected on August 23rd. No reaction ensued.

Re-infested on September 15, 1910, with 20 brown nymphæ (Ref. No. 364). All 20 ticks were found attached on the following day. No reaction followed.

Re-infested on September 30, 1910, with brown nymphæ (Ref. Nos. 355 and 356). On the following day 14 ticks were found attached. No reaction ensued.

Re-infested on October 20th with 6 brown adults, originating from Natal (Ref. No. 349). These adults were found fast on October 21st. The heifer contracted East Coast Fever and died on November 27, 1910.

2. Heifer 911 had been previously used for inoculation purposes in connection with the artificial transmission of East Coast Fever, but with negative results.

Infested on August 17th with a number of brown nymphæ (Ref. No. 319). About 70 ticks were found fast the following day, and the engorged nymphæ were collected on August 23rd. No reaction followed.

Re-infested on September 15th with brown nymphæ (Ref. No. 364). 20 ticks were found attached the following day. No reaction ensued.

Re-infested on September 30, 1910, with brown nymphæ (Ref. Nos. 355 and 356). On October 1st, 20 ticks were found fast. No reaction ensued.

Re-infested on October 20, 1910, by the infestation of 6 brown adults, origin Natal (Ref. No. 349). The heifer contracted East Coast Fever and died on November 6, 1910.

3. Heifer 1014 had been previously used in connection with the artificial transmission of East Coast Fever, but with negative results.

Infested on August 17, 1910, with brown nymphæ (Ref. No. 319). 24 ticks were found fast the following day, and the engorged nymphæ dropped on August 23, 1910. No reaction resulted.

Re-infested on September 15, 1910, with 20 brown nymphæ (Ref. No. 364). 20 ticks were fast on September 16, 1910. No reaction ensued.

Re-infested on September 30, 1910, with brown nymphæ (Ref. Nos. 355 and 356). 10 ticks were found fast the following day. No reaction followed.

Re-infested on October 20, 1910, by the infestation of 6 brown adults, origin Natal (Ref. No. 349). Heifer 1014 contracted East Coast Fever and died on November 21, 1910.

4. Cow 1068 had been previously used in connection with the artificial transmission of East Coast Fever, with negative results.

Infested on August 17, 1910, with brown nymphæ (Ref. No. 363). On the following day about 70 were found fast. The engorged nymphæ were collected on August 23, 1910. No reaction ensued.

Re-infested on September 15, 1910, with brown nymphæ (Ref. No. 364). 20 ticks were found attached the following day. No reaction resulted.

Re-infested on September 30, 1910, with brown nymphæ (Ref. Nos. 355 and 356). 14 ticks were fast the next day. No reaction.

Re-infested on October 20, 1910, with 6 brown adults, origin Natal (Ref. No. 349). 5 adults were fast the next day. This cow contracted East Coast Fever and died on November 21, 1910.

(b) BROWN NYMPHÆ (Ref. Nos. 363 and 373).

5. Ox 1037 had been previously used for the artificial transmission of East Coast Fever, but with negative results.

Infested on September 5, 1910, with brown nymphæ (Ref. No. 319). 27 ticks were found to be attached the following day. No reaction ensued.

Re-infested on January 30, 1911, with 20 brown nymphæ off Heifer 909 (Ref. No. 373). 20 ticks were found fast the following day. Ox 1037 contracted East Coast Fever and died on February 22, 1911.

(c) BROWN NYMPHÆ (Ref. Nos. 363, 364, and 349).

6. Ox 1046 had been previously used for the artificial transmission of East Coast Fever, but with negative results.

Infested on September 5, 1910, with brown nymphæ (Ref. No. 319). 28 ticks were found attached the following day. No reaction developed.

Re-infested on October 20, 1910, with 10 brown nymphæ (Ref. No. 364). On the following day 8 ticks were found fast. No reaction followed.

Re-infested on November 16, 1910, with 4 brown adults, origin Natal (Ref. No. 349). 3 adults were fast the following day. No reaction ensued.

Re-infested on December 8, 1910, with 6 brown adults off East Coast Fever animal 1053 (Ref. No. 411), and re-infested six days later with 2 brown adults off 1053 (Ref. No. 411) and 2 brown adults off Heifer 1111 (Ref. No. 426). 4 ticks of the first batch and 1 tick of the second batch were found fast on the day after infestation. Ox 1046 contracted East Coast Fever and died on January 8, 1911.

(d) BROWN NYMPHÆ (Ref. Nos. 363 and 364).

7. Ox 1043 had been previously used for the artificial transmission of East Coast Fever, with negative results.

Infested on September 5, 1910, with brown nymphæ (Ref. No. 319). 33 ticks were found attached the following day. No reaction resulted.

Re-infested on October 20, 1910, with 10 brown nymphæ (Ref. No. 364). 6 nymphæ were fast the following day. Ox 1043 contracted East Coast Fever and died on November 18, 1910.

8. Heifer 1017 had been previously used in connection with the artificial transmission of East Coast Fever, but with negative results.

Infested on September 5, 1910, with brown nymphæ (Ref. No. 363). 42 ticks were found attached the next day. No reaction ensued.

Re-infested on October 20, 1910, with 10 brown nymphæ (Ref. No. 364). All 10 ticks were fast the following day. A fever reaction set in and Heifer 1017 died of East Coast Fever on November 9, 1910.

(e) RHIPICEPHALUS APPENDICULATUS (Ref. Nos. 364, 349, 411, and 426).

9. Heifer 1090 had not been used previously in any experiment, and was purchased in an area free from East Coast Fever.

Infested on October 20, 1910, with 10 brown nymphæ (Ref. No. 364). On the following day 6 nymphæ were found fast, but no reaction developed.

Re-infested on November 16, 1910, with 4 brown adults (Ref. No. 349), origin Natal. 2 adults were fast the following day, but no reaction ensued.

Re-infested on December 8, 1910, with 6 brown adults off Heifer 1053 (Ref. No. 411). 5 ticks were attached the following day.

Re-infested on December 14th with 2 brown adults off Heifer 1053 (Ref. No. 411) and 2 adults off 1111 (Ref. No. 426). 2 adults were fast the following day. A reaction set in, and the animal died of East Coast Fever on December 27, 1910.

(f) RHIPICEPHALUS APPENDICULATUS (Ref. Nos. 363, 364, and 349).

10. Heifer 1082, an animal which had been born in an area free from East Coast Fever and had not been used previously.

Infested on August 30, 1910, with brown nymphæ (Ref. No. 319). 23 ticks were found attached on September 6, 1910, but no reaction followed.

Re-infested on September 15, 1910, with 20 brown nymphæ (Ref. No. 364). No reaction followed.

Re-infested on October 20, 1910, with 6 brown adults, origin Natal (Ref. No. 349). On the following day 5 adult ticks were found fast. A reaction set in, and the animal died of East Coast Fever on November 25, 1910.

(g) RHIPICEPHALUS APPENDICULATUS, nymphæ (Ref. No. 364).

11. Ox 1050 had been previously used in connection with the artificial transmission of East Coast Fever, without results.

Infested on October 20, 1910, with 10 brown nymphæ (Ref. No. 364). 7 ticks were found attached the following day. A reaction set in, and the ox died on November 21, 1910, of East Coast Fever.

Results.

RHIPICEPHALUS APPENDICULATUS, nymphæ (Ref. No. 319).

These ticks failed to transmit the disease in every instance, and the 9 animals which were infested were proved subsequently to be susceptible to East Coast Fever. The larvæ hatched out on April 26, 1910, and became infected at the end of May and beginning of June, that is, about 5 to 6 weeks after they had hatched. They moulted into nymphæ on August 5th, viz., about 2 months after they had become replete on Heifer 1013. The shortest moulting period in this stage is 16 days in the summer. They were placed on the susceptible animals as follows:—

Heifer	627	infested on August 17, 1910		20	ticks were fast
	911	„ „		70	„
	1014	„ „		24	„
	1068	„ „		70	„
	1082	„ August 30, 1910		20	„
	1037	„ September 5, 1910		27	„
	1046	„ „		28	„
	1017	„ „		42	„
	1043	„ „		33	„

That is to say, 12 days, 25 days, and one month after they had moulted.

RHIPICEPHALUS APPENDICULATUS, nymphæ (Ref. No. 364).

These ticks failed to transmit the disease in seven instances out of ten, the three positive cases being Cattle Nos. 1043, 1017, and 1050. These ticks hatched on April 26, 1910 (the same as batch No. 363, belonging to the same original lot), and were placed on Heifer 908 on August 12th, namely, about 107 days after they had hatched. They moulted into nymphæ on September 10, 1910.

They were placed on the following animals:—

Heifer 627 on September 15, 1910, and 20 became attached			
911	„	20	„
1068	„	20	„
1050 (died)	„	7	„
1082	„	20	„
1014	„	20	„
1046 on October 20, 1910		8	„
1043 (died)	„	6	„
1090	„	6	„
1017 (died)	„	10	„

Of the animals infested on September 15th one died, and of the animals on October 20th two died. It is remarkable that in this instance the minimum number of ticks (6 and 10) transmitted the disease.

RHIPICEPHALUS APPENDICULATUS, nymphæ (Ref. Nos. 355 and 356).

These ticks failed to transmit the disease in four instances when used together for the purposes of infestation.

The larvæ of batch 355 hatched on April 26, 1910, and when they were 79 days old were infected with East Coast Fever; they moulted into nymphæ on September 1, 1910.

Larval ticks number 356 hatched on April 18, 1910, and when 95 days old were infected with East Coast Fever; they moulted into nymphæ on August 15, 1910.

They were placed on the following cattle:—

627 on September 30, 1910, and 14 became attached			
911	„	20	„
1014	„	10	„
1068	„	14	„

RHIPICEPHALUS APPENDICULATUS, adults (Ref. No. 349).

These ticks transmitted the disease to five animals out of seven. The engorged nymphæ were collected off sick cattle in Natal on June 29, 1910, and moulted into adults at the laboratory on September 11, 1910, or 74 days after collection.

They were used for the infestation of—

Cattle	627 on October 20, 1910		3	became attached
	911	„ „		6 „
	1014	„ „		6 „
	1068	„ „		5 „
	1082	„ „		5 „
	1046	„ „		3 „
	1090 on November 16, 1910		2	„

RHIPICEPHALUS APPENDICULATUS, nymphæ (Ref. No. 373).

These ticks transmitted the disease to one animal (1037). As larvæ they were infected on Heifer 909 on January 7, 1910. They moulted into nymphæ on January 20, 1911, and were used on January 30, 1911, when 10 days old.

They were placed on—

Heifer 1037 on January 30, 1911, and 20 became attached.

RHIPICEPHALUS APPENDICULATUS, adults (Ref. Nos. 426 and 411).

Batch No. 426 transmitted the disease to two animals, 1040 and 1090. They were infected as nymphæ on October 26, 1910, and moulted into adults on November 26, 1910.

Batch No. 411 were infected as nymphæ on October 18, 1910. They moulted into adults on November 21, 1910.

The adults of batch 426 were 18 days old when used, and the adults of batch 411 were 18 days old when used.

Ticks Ref. No. 411 were placed on Heifer 1046 on December 8, 1910, of which 4 became attached, and on December 14th Ref. Nos. 411 and 426 were infested, of which 1 became attached. Ref. No. 411 undoubtedly transmitted the disease. Ref. No. 411 were also placed on Heifer 1090 on December 8, 1910, of which 5 became fast, and on December 14, 1910, 411 and 426 were infected, of which 2 became fast. The last batch transmitted the disease.

EXPERIMENT No. 3.

TO NOTE WHETHER BROWN ADULTS WHICH WERE FEEDING AS LARVÆ ON CATTLE SUFFERING FROM EAST COAST FEVER, AND WHICH PROVED TO BE VIRULENT AS NYMPHÆ, WILL TRANSMIT THE DISEASE TO ADULTS AFTER HAVING PASSED THEIR NYMPHAL STAGE ON A RABBIT.

Particulars of the Ticks.

1. RHIPICEPHALUS APPENDICULATUS (Ref. No. 342).

The larvæ originated from mothers collected in Natal on January 12, 1910; the eggs were laid on January 18th, and the larvæ hatched on February 18, 1910, and were placed on 923 on March 20, 1910 (Ref. No. 268), at a time when the parasites of East Coast Fever were frequently noted in the blood. The engorged larvæ were collected on March 23, 1910, and hatched out in due time. One lot was placed on Cow 596 on April 7, 1910, and transmitted the disease (*vide* Experiment No. 1 (a)). A second lot was placed on a rabbit, and the engorged nymphæ were collected from April 13 to 29, 1910.

2. RHIPICEPHALUS APPENDICULATUS (Ref. No. 309).

The larvæ originated from mothers collected in Natal; the eggs were laid on December 24th and the larvæ hatched on January 30, 1910; they were placed on Calf 700 on February 27, 1910 (*vide* Experiment No. 1 (b)). The engorged larvæ were collected during the period when the parasites of East Coast Fever were frequently met with in the blood. These engorged larvæ hatched on March 17, 1910, and the nymphæ were placed on Calf 917 (*vide* Experiment No. 1 (c)), and transmitted the disease. Another lot was placed on rabbits, and the engorged nymphæ were collected from June 3 to 7, 1910. They moulted on May 4, 1910.

Testing the Ticks.

1. Heifer 1059 was infested on August 29, 1910, with adult brown ticks (Ref. No. 342) of the first rabbit. A re-infestation was made on September 14th with ticks of the second rabbit (Ref. No. 309). On September 19, 1910, 26 ticks were found attached. No reaction followed; the heifer eventually died of East Coast Fever at "Burnside."

2. Heifer 1019 was infested on June 23rd with 20 brown adults of the first rabbit (Ref. No. 342). On June 28th it was re-infested with 20 brown adults of the second rabbit (Ref. No. 309). On the following day 8 adults were found attached. On July 15th and 20th and on August 3rd and 20th this heifer was re-infested with brown adults of the first rabbit

(Ref. No. 342). On July 28th 9 ticks were found fast. On August 4th no ticks could be seen. No reaction followed, but the animal eventually died at "Burnside" of East Coast Fever.

CONCLUSION.

The infestation of two susceptible heifers with adult brown ticks which in their larval stage had been feeding on cattle suffering from East Coast Fever (and as nymphæ proved infective for cattle) did not convey East Coast Fever as adults after they had passed their nymphal stage on a rabbit.

EXPERIMENT No. 4.

TO NOTE WHETHER BROWN ADULTS WHICH FED AS NYMPHÆ ON CATTLE IMMUNE TO EAST COAST FEVER WILL TRANSMIT THE DISEASE TO SUSCEPTIBLE CATTLE.

Particulars of Ticks.

Heifer 914 had been infested with brown nymphæ (Ref. Nos. 268, 335, and 309) on June 28, 1910, and contracted East Coast Fever, from which it recovered. Plasma bodies were found in the glands and *Theileria parva* in the blood. The heifer showed a typical East Coast Fever reaction, and by the end of May had completely recovered.

History of Ticks used to Infest the Immune Heifer.

Brown nymphæ (Ref. No. 298) off Heifer 868, susceptible to East Coast Fever, were placed on Heifer 914 on September 2, 1910. These nymphæ engorged and dropped on September 6 and 7, 1910.

Testing the Ticks.

They moulted into adults on October 19, 1910, and on December 15th these adult ticks were placed on Heifer 1021. The following day the ticks were found fast. No reaction followed. Heifer Calf 1130 was infested on January 6th with ticks of the same batch as above (brown adults, Ref. No. 298), and the following day they were found attached. No reaction followed.

CONCLUSION.

The infestation of two susceptible cattle with adult brown ticks which had passed their nymphal stage on a heifer immune to East Coast Fever did not convey the disease. This observation supports some conclusions made as far back as 1903.

SUMMARY OF CONCLUSIONS.

In the foregoing experiments it has been proved—

1. That brown tick imagines which as larvæ had become infected with East Coast Fever and which had transmitted the disease in their nymphal stage were no longer infective for susceptible cattle. Four batches of ticks proved their infectivity in the nymphal stage on eight animals, but in their adult stage failed to transmit the disease to two susceptible animals.

2. Ticks belonging to the same batches which were feeding on two animals rendered immune to East Coast Fever by inoculation in the nymphal stage did not transmit the disease in their adult stage to six animals, thus proving that the brown tick which has become infected in one stage cleans itself in the following stage by feeding on an immune or susceptible animal.

3. Ticks which became infected with East Coast Fever in their larval stage, and which passed their nymphal stage on a rabbit, did not prove to be infective in their adult stage for susceptible cattle. This conclusion bears out that given above (2), showing that a tick loses its infectivity the first time it feeds on an animal susceptible or immune to East Coast Fever.

4. Clean or infective ticks feeding on an animal which has recovered from an attack of East Coast Fever do not transmit the disease in their next stage. This conclusion is in support of experiments undertaken eight years ago (*vide* Annual Report of the Government Veterinary Bacteriologist, 1904–05).

5. It has been demonstrated that certain batches of ticks collected at the same time and which fed under similar conditions do not transmit the disease in their next stage, even when infested in great numbers and on numerous animals. Other batches of ticks reared in exactly the same way and under similar conditions only infected a few animals, whilst again other tricks proved infective in almost every instance even when a minimum number were used.

It is difficult to give an explanation of this fact, but it is quite likely that outside conditions have some influence. The ticks which did not transmit the disease were bred during the coldest time of the year. This may perhaps explain the fact that during the winter months the infection in the field is not so active as during the summer months, remembering at the same time that during the winter months tick life is generally weaker than in the summer.